CHEMICAL DEVELOPMENTS *in* THYROIDOLOGY

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Edited by

WILLARD O. THOMPSON, M.D.

Clinical Professor of Medicine
University of Illinois College of Medicine
Managing Editor, Journal of Clinical Endocrinology
Chicago, Illinois

CHEMICAL DEVELOPMENTS *in* THYROIDOLOGY

WILLIAM T. SALTER, M.D.
Professor of Pharmacology
Yale University School of Medicine
New Haven, Connecticut

CHARLES C THOMAS · PUBLISHER
Springfield · Illinois · U.S.A.

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INTRODUCTION

It has been said that in this century the mathematicians are becoming philosophers; the physicists mathematicians, the chemists physicists, and the physiologists chemists. In the field of endocrinological investigation, formerly the province of the old-time mammalian physiologist, this drifting of the sciences has also made itself apparent. The past decade has seen great strides in the study of thyroid physiology and thyroid disease. Most of this progress, however, has been made with tools and methods that are primarily chemical in character. Indeed, the investigative approach has even drifted into the field of nuclear physics It is the purpose of this essay to describe some of these interesting developments.

To this end, attention will be focused upon four main types of study, which can be catalogued as follows:

(1) Short-cut synthesis of the thyroid hormone by methods which imitate nature.

(2) The development of blocking agents which impede the natural synthesis.

(3) Studies of the peripherally circulating hormone.

(4) The application of radio-iodine to problems in physiology and therapy.

None of these trends is yet completed and it may be some time before the whole story can be written of each. Nevertheless, already so much material has accumulated that the inner working of the thyroid gland is becoming much more comprehensible. Moreover, these trends interdigitate so well

SYNTHESIS OF THE THYROID HORMONE

EVER SINCE Baumann (13) discovered in 1895 that the thyroid contained iodine and, as he correctly surmised, its essential principle likewise, attempts have been made to produce thyroid activity by incorporating iodine into organic materials. Baumann himself (14) attempted to increase the activity of the thyroid globulin by iodinating it further, but without success. Later other investigators tried the same experimental approach. Notable among these was Oswald, who about 1911 published a long series of investigations on iodinated proteins (82) (83) (84). He noted that there were several steps in the iodination of the protein. He noted, further, that some of the iodine was bound to nitrogen, probably as iodohistidine. He was unable, however, to demonstrate the production of endocrinological activity in these proteins. For this reason interest in the iodinated proteins ultimately lapsed. Nevertheless, the general hypothesis lay in the back of biochemists' minds, namely, that by introducing iodine into protein a remarkable endocrine activity might be produced.

The essential question was how this remarkable property could arise; and, in particular, what was the chemical mechanism by which the living gland effected such a synthesis. From time to time, this problem recurred and several investigators thought they had produced weak endocrine potency through the iodination of impotent material. The most outstanding of these preliminary investigations was that of Abelin (2) (3), whose iodinated proteins hastened the metamorphosis of tadpoles. When Lerman and Salter (66) showed that the iodination of serum protein could yield in

that a new biochemical era in thyroidology seems to have arrived.

To be sure, the dawn of this era had been foreshadowed even before the Global War broke. For instance, nearly a decade ago the author summarized the existing knowledge of thyroid function (93) and emphasized the "Endocrine Function of Iodine" (94). The humble beginnings of that time, however, have now blossomed and borne fruit far beyond his highest expectations. For example, the only pure goitrogenic agents considered in that treatise were thiocyanate and certain cyanides. Furthermore, the use of radio-iodine in human therapeutics was mentioned only as a dream. It is the purpose of this essay to describe these recent advances and to hazard some evaluation of them.

yield of hormone. Among these are the acidity of the medium, the temperature of incubation, the degree of aeration of the mixture, the rapidity of stirring, and the extent of the iodination. In addition, some evidence has been adduced which suggests that the reaction is catalyzed by compounds of manganese. Before discussing in detail the influence of these variables, it should be stated at once that these studies have confirmed amply the original hypothesis of Harington and Barger (44). Those investigators suggested *that thyroxine should arise in nature by the conjugation* of two molecules of diiodotyrosine. Now that the process has been demonstrated *in vitro*, the time is ripe to extend the basic procedure along biosynthetic lines.

The conditions under which this synthesis occurs in the laboratory involve a mild oxidation and the elimination of one of the alanine side-chains of the two diiodotyrosine molecules. This fact is of considerable biological interest because it suggests the mechanism by which the gland itself operates in the elaboration of this specific hormone.

As to the optimal conditions under which iodinated proteins produce maximal yield, the following are those indicated by Reineke's studies (108) of the process The pH should lie between 6.8 and 8.0. The temperature during iodination should be kept between 30 and 45° C. The medium should be buffered with sodium bicarbonate while the iodine is added. Approximately four to five atoms of iodine are required per mol. of tyrosine. A subsequent incubation with stirring for eighteen to twenty hours is then carried on at a temperature between 50 and 100° C. It seems likely, also, that the process can be expedited by some catalyst: possibly copper, tin or zinc. When these conditions are followed as closely as possible, the production of crude thyroxine is approximately three percent, if manganese tetroxide is used as a catalyst.

a few minutes a preparation which relieved classical human myxedema in striking fashion, there no longer remained any doubt that iodinated protein could indeed contain effective hormonal activity. Moreover, quite recently, Dvoskin (32) has relieved experimental myxedema by intramuscular injection of elementary iodine in solution. In 1936 Ludwig and von Mutzenbecher (69) iodinated casein and from the hydrolysate thereof isolated crystalline racemic thyroxine, as well as crystalline diiodotyrosine and (probably) crystalline monoiodotyrosine The isolation of the crystalline thyroxine was confirmed by Harington and Pitt Rivers (46) and by several other investigators in America. Consequently there no longer was any doubt that the genesis of thyroxine could occur during the iodination of protein. The problem then resolved itself in two separate developments. The first of these was to improve the yields of the original process. The second was to unravel the chemical mechanism by which this rapid synthesis of thyroid hormone occurred.

In the original procedure the yield of thyroxine obtained by Ludwig and von Mutzenbecher was about one-tenth of one per cent in terms of the total iodine involved. Subsequently Block (16) obtained a sample of crystalline thyroxine from pure iodotyrosine which had been incubated in an alkaline medium This yield, however, was only about one-tenth of one percent. Such small yields were disappointing, even though the qualitative mechanism of production had been demonstrated adequately. Thereupon there ensued a series of experiments designed to increase the efficiency of this synthetic process.

The outstanding work along these lines was conducted by Turner and Reineke (109), (110), (88), and was concerned mostly with the iodination of casein A systematic study of the reaction was made. Such observations showed that there are several factors which influence appreciably the

yield of hormone. Among these are the acidity of the medium, the temperature of incubation, the degree of aeration of the mixture, the rapidity of stirring, and the extent of the iodination. In addition, some evidence has been adduced which suggests that the reaction is catalyzed by compounds of manganese. Before discussing in detail the influence of these variables, it should be stated at once that these studies have confirmed amply the original hypothesis of Harington and Barger (44). Those investigators suggested that thyroxine should arise in nature by the conjugation of two molecules of diiodotyrosine. Now that the process has been demonstrated *in vitro*, the time is ripe to extend the basic procedure along biosynthetic lines.

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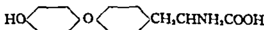
This result has been confirmed both by biological and chemical assays. However, the actual yield of crystalline thyroxine on isolation is considerably lower because of inherent losses in the process of isolation. In fact, yields of d, l-thyroxine higher than 0.5 percent have not yet been obtained. *Moreover, even to obtain such yields it has been necessary to use the classical isolation procedure which in*volves alkaline hydrolysis with barium hydroxide. This alkaline hydrolysis racemizes the natural optically active thyroxine: so that the final product is a racemic mixture. Because biological assays indicate that pure l-thyroxine has twice the potency of the d, l-mixture, it would be desirable to obtain thyroxine solely in the form of the levorotatory isomer. This preparation has in fact been performed by heating the iodinated protein with sulfuric acid. The resulting hydrolysis, however, is not so efficient and the recoveries of pure l-thyroxine by this process are considerably lower than those obtained from the alkaline baryta. Indeed, even allowing for the higher activity of the levorotatory isomer, the total activity so obtained is less than that of the d, l-combination. So much for the isolation of the hormone from iodinated protein.

Of considerable theoretical interest, moreover, is the fact that crystalline thyroxine can be synthesized from pure diiodotyrosine. This finding simplifies considerably the chemist's problem, namely, by what mechanism diiodotyrosine turns into thyroxine The importance of this question for the biochemist lies in the fact that it gives a clue to the internal physiology of the thyroid itself.

Because the synthesis of active hormone from casein can be pursued on a large scale, agriculturists are interested in the possibility that it might be used in animal husbandry Consequently, a number of experiments are now in progress to determine how profitably such material may be employed

by dairies and farmers. In England (15) (85) a series of experiments has been conducted with various starting materials, including a powder obtained from "ground nuts." In the United States most of the work has been limited to iodinated casein. Preliminary results indicate that apparently some increased production of dairy products can be effected with this material. For example, in laying hens the synthetic material appears to counteract somewhat the decline of egg production which occurs during hot weather. Similarly young hogs have grown a little more rapidly and chickens have shown somewhat faster rates of body growth and marked increases in feather growth. In lactating animals, e.g., cows and goats, a consistent increase has been shown in the production of milk, and particularly of the milk fat. It remains to be learned, however, whether the cost of such treatment is justified by the increased production. This cost, obviously, must be calculated in terms of both the medication and the increased metabolic turnover which ensues. The answer is by no means clear as yet.

Apart from these practical considerations, however, the pure scientist and the clinical investigator are more interested in the implications which the reactions thus demonstrated may have for physiology and pathological physiology. This problem has been considered by a number of investigators using various chemical approaches. One of the earliest and most fruitful of these was the study of Johnson and Tewkesbury (57). They started with the knowledge that certain essential features were needed for thyroid activity. The chief of these, as outlined by Harington (42), was the presence of the organic configuration known as "thyronine." Its structure is illustrated in the following formula.



Many studies have shown that this general framework in the molecule is relatively essential for potency. For example, through the synthesis of "iso-thyroxine," i.e., β, β-di (3, 5-diiodo-4-hydroxyphenyl) α-amino-propionic acid, it was shown that the mere presence of all the essential atoms and chemical groups was not enough. As indicated in the formula just given, this compound contains two phenyl groups directly linked to carbon instead of in the classical diphenyl-ether combination. The test material, synthesized by Harington and McCartney (45), proved to be inert physiologically. Therefore, a considerable part of the present problem consists in determining how two molecules of diiodotyrosine can combine to form thyroxine. As a matter of fact, it is not necessary to have four iodine atoms in thyroxine in order to achieve activity. It was shown, for example, by Anderson, Harington and Lyon (5) and by Lerman and Salter (67) that diiodothyronine is fully effective if given in adequate doses. Nevertheless, the activity of this compound is only one-twenty-fifth that of thyroxine itself. How, then, does the diphenyl-ether structure arise from two molecules of diiodotyrosine?

As a tentative answer to this question Johnson and Tewkesbury (57) proposed an oxidative mechanism. They made certain assumptions based upon the work of Pummerer (86), who studied the oxidation of o- and p-substituted phenols in alkaline solution. Pummerer had adduced evidence that a quinol-ether intermediate might be formed under such circumstances. Accordingly, Johnson and Tewkesbury oxidized 3,5-diiodotyrosine with hypoiodous acid (HOI). Under these circumstances in addition to the quinol-ether, ammonia and puruvic acid should be formed, as indicated in Fig 1.

These two substances were actually identified in their reaction mixture.



FIG. 1. Hypothetical conversion of diiodotyrosine to thyroxine. After Johnson and Tewkesbury, *Proc. Natl. Acad. Sci. U.S.*, 28:74, 1942.

This line of attack was taken up by Harington (43) who was able to increase the yield of the thyroxine formed. Starting with the diiodotyrosine in a two-phase system, he oxidized it with dilute chromic acid and obtained a gross yield of 1.63 percent, as compared with the earlier recovery of 0.23 percent attained by von Mutzenbecher. The reaction proceeds best in an alkaline solution, near pH 10. In addition, some elevation of temperature is advantageous. The greatest aid in recovery, however, Harington found was the use of a second phase of butyl alcohol. This phase removes the thyroxine produced in the reaction mixture as fast as it is formed and in this way drives the reaction further to completion. It also spares the product from further oxidation by the chromic acid. Of special interest was the fact that starting with d-tyrosine, Harington was able to produce d-thyroxine; and from l-tyrosine, l-thyroxine.

To the organic chemist these results are exciting, because only a few years ago it was quite problematical how the very stable diphenyl-ether linkage could be established. Harington has adopted tentatively the hypothesis of Westerfeld and Lowe (114), who showed that a quinoid compound could react with itself to form a sort of dimolecular quinone. Thus the mechanism of the reaction visualized would be a chemical conjugation of two substituted phenols

These observations constitute an excellent background for the mechanisms of biosynthesis as they occur in the gland. This aspect of the problem at present is only just being investigated, but already certain fundamental considerations are clear. These have been clarified greatly through the use of the thyroid blocking agents which check the synthesis of the hormone at one or more stages The first problem to be solved is how the gland can trap iodide at very high differential concentration between the gland itself and the medium in which it is bathed. The ratio may vary from 10-fold

to 1000-fold (96) under various circumstances. Salter (98) has adduced evidence that the iodide within the gland exists in two forms, both of which are recoverable ultimately as ionized iodine. Part of this iodide in the gland exists in the ordinary free ionized form. This moiety is designated as I_F. The other part is bound to a colloidal system, from which it can be freed with agents which coagulate protein. This fraction is known as bound iodide and designated as I_B. The "bound iodide" can not be regarded as permanently incorporated into the metabolic path leading to the formation of the thyroid hormone.

It must be transformed into diiodotyrosine before this process is fully launched. Obviously this process involves the temporary production of iodine in a state equivalent to, although not necessarily identical with, elementary iodine. An oxidation process then can occur which transforms tyrosine to diiodotyrosine.

That an oxidation-reduction process actually is involved is indicated by experiments with the thyroid blocking agents which have a redox potential lower than a certain critical value. Substances with molal electrode potentials at this level need not necessarily be thyroid blocking agents; but, as yet, no thyroid blocking agent in the thiouracil series has been discovered which does not show its molal electrode potential within a certain physico-chemical range (68). Whether or not the natural iodase system is simple or complex remains unknown For example, it might conceivably consist of an iodinase and a periodase complex. Thus, the iodinase system would combine with iodide and so produce a compound analogous to a peroxide or hypoiodite. Thereupon the periodase might conduct the final oxidation of tyrosine to diiodotyrosine. When this enzymic system is blocked through the use of a thiouracil derivative, the synthesis of diiodotyrosine fails to occur. Presently mention will

be made of a different type of block produced by thiocyanate.

Salter (98) has shown that different thyroid blocking agents act at different stages in the synthesis of thyroxine. It seems probable, therefore, that a separate enzyme system deals with the conversion of diiodotyrosine to thyroxine. The exact anatomical site or sites of this conversion, however, remain undetermined. Perhaps the reaction occurs within the cytoplasm of the thyroid follicular cells. It might also occur within thyroglobulin already stored in the thyroid follicle but as yet not possessing full endocrine potency. In either case, a careful distinction should be made between the synthesis of active hormone and its release. It is very clear now that although thiouracil prevents the synthesis of new hormone, it does not retard to any observable extent the continuous release of pre-existing stores of the hormone, as represented by the follicular colloid or thyroglobulin. Indeed under certain circumstances, as in chickens, it is possible by the use of thiouracil to produce large masses of thyroid colloid which is quite inert. The problem of the release of thyroid hormone, therefore, becomes a separate problem in itself and one presumably related to the action of the thyrotropic hormone. This question will be touched on later.

The rate at which thyroid hormone is released and the mechanism of its release from the gland is, of course, extremely important from the standpoint of the general metabolism of the organism Many morphologic approaches to this problem have been made in the past, most of them based upon the supposition that the thyroglobulin or colloid travels *per se* from its central location in the follicular lacuna into the bloodstream. Some earlier observers even reported that they could see small droplets of the colloid migrating between follicular cells into the bloodstream (113).

In recent years, however, the peculiarities of protein chemistry have made themselves felt in this field. It has been suggested, for example, that the thyroglobulin first must be autolyzed through proteolytic enzymes. The resulting peptides (92), (99) would then be small enough to diffuse from the central follicles through the follicular membranes into the bloodstream. In confirmation of this hypothesis, de Robertis (30) demonstrated that, within fifteen minutes after the administration of thyrotropic hormone, the proteolytic activity of the follicular contents increased markedly even though no significant change in hydrogen ion activity occurred. Such observations lend credence to the supposition that the large aggregates of thyroglobulin, perhaps 750,000 in molecular size, are held behind the follicular walls until such time as they are broken up by enzymic action. The small fragments of the colloid then are able to stray from their storage place into the surrounding perifollicular vascular network. Such a hypothesis also accounts for the observations of Lerman (64) and others that ordinarily no thyroglobulin can be demonstrated in the circulating blood This statement is not true, however, when the gland itself is subject to such trauma that gross extravasation of thyroglobulin into the thyroid veins can be demonstrated. Such observations, obviously, are not of physiological significance; and consequently Lerman (65) has interpreted his results as meaning that thyroglobulin *per se* does not travel in the systemic circulation Whether the lymphatics of the thyroid carry off hormone under physiological conditions is still unknown. Indeed, we are not even sure whether hormone, either in protein or in peptide form, travels through the thyroid lymphatics. Earlier observations by Carlson and his associates (20) may be interpreted on the basis of traumatic displacement of the thyroid stores.

A further aspect of the question of the valence of iodine,

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a considerable amount of the thyrotropic hormone is inactivated by exposure to the thyroid tissue. This tropic hormone, however, can be reactivated by treatment with goitrogenic agents like thiouracil which are reducing agents. When, however, sodium iodide is added to the medium in which slices of thyroid tissue are bathed, the inactivation of the thyrotropic hormone dissolved in the tissue culture fluid is prevented. This finding raises the question whether a similar phenomenon might be demonstrated in animals. Rawson has answered this question by observing that the thyroid of the hypophysectomized rat, which ordinarily responds to injections of thyrotropic hormone, fails to do so if large doses of sodium iodide are administered simultaneously.

This ability of explants of normal human thyroid tissue to inactivate thyrotropic hormone is of considerable interest. Rawson finds that 200 milligrams of explants of normal *thyroid will inactivate three units of thyroid-stimulating hormone* On the contrary, explants of non-toxic nodular goiter do not inactivate tropic hormone, whereas explants of thyroids from treated cases of Graves' disease will inactivate six to seven and one-half units of the hormone. Exposure to slices of normal thyroid tissue will inactivate also the exophthalmos-producing factor of crude pituitary extracts The same technique has been tested by Rawson (87) on slices of normal animal tissue. For example, 150 milligrams of rabbit thyroid, when sliced, inactivated twelve units units of thyrotropic hormone. Control experiments showed, however, that thyroid tissue would not inactivate the gonadotropic principles of a crude pituitary extract. Likewise explants of miscellaneous organs, e.g, liver, spleen, kidney, ovary, testes, pancreas and others, have failed to inactivate the thyrotropic hormone Indeed, the only tissues which behaved like the thyroid were the thymus and lymph nodes. This last finding is of special interest because, as has long

i.e., the state of oxidation of iodine in relation to thyroid metabolism, is raised by the experiments of Albert and Rawson (4). These investigators have shown that elementary iodine inactivates thyrotropic hormone reversibly. If, by some process, an excess of elementary iodine were produced within the gland, the thyrotropic hormone reaching the gland might be iodinated and so made ineffective. If such a process could be expedited by the administration of an excess of iodide to the organism, then an excess of the thyrotropic hormone — occurring, for example, in Graves' disease — might be inactivated. One difficulty with this theory, at the present time, is the general knowledge that several proteins are altered reversibly by iodination. An outstanding example of this feature is insulin (56), which loses its activity on iodination and regains it in large measure if the iodine be removed from the insulin molecule by appropriate methods. Nevertheless, one can not pass over this possible mechanism without giving it due attention.

It is entirely conceivable that, when an excess of iodide is supplied to the gland, an increased amount of "bound iodide," I_B, is formed. This fraction is then on the way towards a state equivalent to elementary iodine. In other words it may not perhaps be elementary iodine itself which inactivates the thyrotropic hormone within the gland. Rather, it may be the *equivalent* of elementary iodine in the form of an enzymic complex equivalent to an iodinase system It is evident that much more fundamental information is needed about the cellular physiology of the thyroid, before a more definite evaluation of these results can be made. Rawson (59) has concluded that the thyrotropic hormone acts upon the follicular cells by donating an essential part of its molecule to the cell. Possibly this reaction involves an oxidative process. The evidence for this is that, when tissue cultures of thyroid tissue are treated with thyrotropic hormone *in vitro,*

their importance as therapeutic agents, they have a great importance as laboratory tools. Through their use it has been possible to dissect the internal economy of iodine so that in a complicated dynamic process, certain parts can be studied separately. Therefore, it will appear presently, as these substances are discussed, that the understanding of their actions is part and parcel of the problem of the biosynthesis of hormones which has just been considered.

been known, lymphatic tissue tends to hypertrophy in exophthalmic goiter.

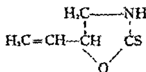
These observations of Rawson suggest a direct relationship between iodine metabolism and the thyrotropic hormone. It still must be admitted, however, that thyrotropic action might be explained by an indirect "vital" influence upon tissue enzyme systems. For example, de Robertis (31) found that, within fifteen minutes to an hour after the administration of the thyrotropic hormone, the protease activity of the follicular contents increased. Furthermore, Rawson (62) observed an increase in mean cell height of 160 percent within twenty-four hours after such an injection. Obviously, profound changes are produced by the thyrotropic hormone both in the economy of follicular cells and in the chemical state of the follicular contents. Therefore, it might still be argued that the effect of thyrotropic hormone primarily involves cell metabolism and cell enzyme systems, rather than a direct effect upon iodine metabolism itself.

If this second point of view were adopted, the finding of Chaikoff (81) that thyrotropic hormone increases the *assimilation of iodine by the thyroid gland might be explained* on the basis that the enzyme systems concerned with the trapping of iodine have hypertrophied. Because of their enlarged capacities, therefore, these enzymes could the more readily seize upon iodide ions as they enter the follicular cell.

Probably the perennial question how iodide favorably affects the course of classical Graves' disease will not be understood until the questions of the preceding paragraphs have been answered fully. An important advance, however, toward the understanding of these problems has been made in the rapid expansion of our knowledge of the goitrogens which has occurred in the last few years. Regardless of

ical confirmation in goiters reported by Barker (112), by Means (75) and by O'Hare (91) in patients receiving thiocyanate treatment over prolonged periods.

Indeed, it is quite possible that most normal people are continuously exposed to the action of goitrogenic substances which occur in their normal diets. In addition to vegetables like cabbage, turnip or rape which have been studied in laboratory animals, Astwood and his associates have found that goitrogenic activity resides in several vegetables and fruits commonly found in the American dietary. Of special interest is the high activity in the yellow turnip or rutabaga (*Brassica napobrassica*) The essential substance as isolated in crystalline form is l-5-vinyl-2-thioöxazolidone. This substance, the formula for which follows, occurs also in the



roots of white turnips and in the seeds of cabbage, kale, rape, rutabaga and white turnip. Its goitrogenic potency in man is close to that of 6-*n*-propyl-thiouracil

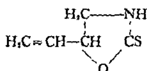
This body of knowledge was brought to a focus and developed by the outstanding investigations of Astwood (6), who finally developed thiouracil and propyl-thiouracil as effective clinical agents in hyperthyroidism His work was confirmed by the observations of Himsworth (52) in London; and now many thousands of cases have been reported from all parts of the globe. There is no question that in most cases of Graves' disease and toxic nodular goiter a very striking effect occurs. The circulating hormonal iodine falls, the basal metabolic rate falls and the concentration of blood cholesterol usually increases What is most important is that the patient is much improved, caloric waste dimin-

THE BLOCKING OF THYROID ACTIVITY

For several decades attempts have been made to check the activity of the thyroid gland by chemical agents. Originally the purpose of such investigations was to find a remedy for Graves' disease which would avoid surgical intervention. One of the first attempts along these lines was that of Abderhalden (1) who had been interested in specific tissue immunity. He had produced specific immune serum against certain organs and hoped by this means to influence the thyroid. This type of material — the so-called anti-hormone — did not prove to be a practicable clinical therapeutic tool. In the laboratory, however, Ivy produced undeniable suppression (78) of thyroid activity by this means. More recently Lerman (65) attempted the same thing in animals and found that rabbits could be made definitely myxedematous by such preparations Other animals, however, were not affected Undeniable investigation of the myxedematous rabbits indicated that their glands had undergone an almost complete atrophy. In short, the product produced was probably not a true anti-hormone. Rather, it had a deleterious action on the gland itself under certain circumstances. In the meantime, Mackenzie, Mackenzie and McCollum (74) had shown that the prolonged administration of sulfaguanidine in animals would produce goiter. Similar responses have been noted also by Hercus (47) and other Australian investigators, who studied rapeseed and its chemical goitrogenic agents. Moreover, Richter and Clisby (89), and Kennedy (60) showed that thiourea derivatives would likewise produce the same effect. These observations received a certain clin-

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This body of knowledge was brought to a focus and developed by the outstanding investigations of Astwood (6), who finally developed thiouracil and propyl-thiouracil as effective clinical agents in hyperthyroidism. His work was confirmed by the observations of Himsworth (52) in London; and now many thousands of cases have been reported from all parts of the globe There is no question that in most cases of Graves' disease and toxic nodular goiter a very striking effect occurs. The circulating hormonal iodine falls, the basal metabolic rate falls and the concentration of blood cholesterol usually increases. What is most important is that the patient is much improved, caloric waste dimin-

ishes, the body weight rises and nervousness disappears. Indeed, many cases seem to be cured permanently of the disease, perhaps one-third of all patients.

The actions of the various goitrogenic agents which have been studied are all qualitatively rather similar. If, for example, rats are treated from birth, definite cretinism will occur under the action of several of these agents. This condition, however, can be prevented if an excess of thyroxine be administered simultaneously. In chicks, in addition to a tremendous enlargement of the thyroid there are marked changes in the body feathers, comb, and wattles. The spurs remain undeveloped, the body skeleton is small, and the joints are hypermobile due to weak muscular development. Ultimately the fat, edematous chick is unable to stand. There are also some secondary findings which remain unexplained as yet. For example, in rats treated in a warm environment the thyroid hyperplasia produced by thiouracil may fail to appear. The goitrogens also check amphibian metamorphosis in species like *rana pipiens*. There are some species differences. For example, the very young guinea-pig responds much less effectively than does the young rat. Likewise, certain goitrogens are effective only if the iodine in the environment is low. In this class are methyl cyanide and thiocyanate On the contrary, other goitrogens, e.g., aminobenzene and thiourea, are goitrogenic even in the presence of a rather high iodine intake.

A great deal of active work on the action of these agents is now in progress in laboratories all over the world. The present investigations seem to be concerned chiefly with two problems The first of these is the cataloguing of various types of compounds which exhibit goitrogenic action. Part of the interest in this work lies in the possible clinical utility of such drugs. In the present article, however, another aspect of this work will be emphasized, namely the chem-

ical structure and properties of those drugs which are found to be goitrogenic. Already literally hundreds of compounds have been screened in animals (7), (72), (73). Among the sulphur-containing compounds which are effective are 2-thiouracil, thiobarbituric acid and thiourea. Of the many other compounds found to have goitrogenic action only a few can be mentioned: benzimidazole-2-thiol, pyridine-2-thiol, thiazoline-2-thiol, 5, 5-diethyl-2-thiobarbituric acid, 5-benzal-2-thio-hydantoin, *sym*-diethyl thiourea. Goitrogenic activity is by no means confined to compounds containing sulphur. Aniline derivatives like the sulfaguanidines, p-, m-, and o-amino-benzoic acids, p-amino phenylacetic acid and p-amino acetanilid were also found to be effective. In addition, Stanley and Astwood found that the activity of l-methyl-2-mercaptoimidazole *in man* is a hundred times as active as thiouracil McGinty (73), (19) has described many other compounds which have been screened for possible goitrogenic effects. Of these substances 5-aminothiadiazole-2-thiol and 3-(phenylaminomethyl)-thiazolidine-2-thione are about as potent as thiouracil and show low toxicity in rats. It is amusing that the complexity of the many combinations and permutations of the compounds thus far tested has forced investigators into a systemic codification which simplifies the recording of the results It is not clear that these compounds all necessarily operate by the same mechanism At the present time, however, most of the investigation is being pushed with sulphur-containing compounds like thiouracil, in order to gain some idea of the mechanism thereof.

Thus far no definite theory has been evolved which would indicate the probable potency of any projected goitrogenic agent Most of the agents which have been tested thus far fall into two general groups, as shown in Fig. 2. These are, respectively, derivatives of thiourea or of aniline. Williams and his associates (115) have studied the effect of

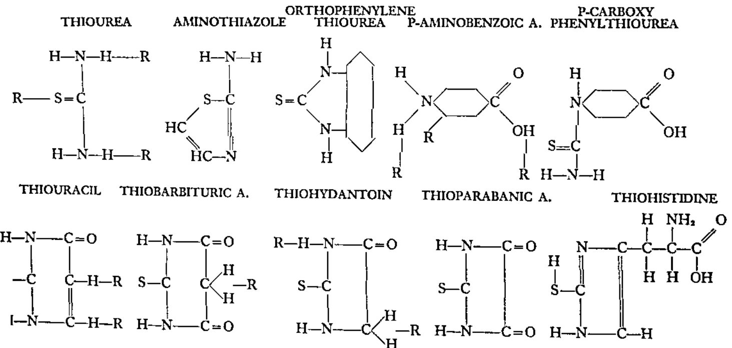


FIG 2. Several characteristic types of synthetic goitrogens. From Williams, R H., and G. A. Kay, *Am J M. Sc.*, 213:198, 1947.

minor structural changes in the two main series of goitrogenic compounds, namely thiocarbonamides and aminobenzenes. They have found that minor structural changes in these compounds may modify the activity considerably, either positively or negatively. For example, the C = S configuration is essential, and even the substitution of other radicals on the sulphur group tends to destroy the potency. Most of the active compounds contain this C = S linkage adjacent to two amino groups, although one of the amino groups may be replaced by a radical containing sulphur or oxygen. The introduction of iodine into either the thiourea or the aminobenzene series apparently does not increase the activity of the compounds.

When the thiourea chain is closed, the effectiveness of the drug is usually increased. Substitution in the sixth position of thiouracil, particularly by shortchain hydrocarbon radicals, tends to increase the potency of the drug This effect of substitution at the sixth position is illustrated by Fig. 3.

Of special interest is the finding by Williams and Kay (116) that when thiouracil and p-aminobenzoic acid are administered simultaneously, a synergistic or potentiating effect is noted. This observation is important because it suggests that an effective way of avoiding toxicity in the clinic may consist in the simultaneous use of two or more goitrogenic agents The same principle has recently been suggested in the case of the sulfonamides by Frisk, Hagerman, Helander, Sjogren (36).

So many of these compounds have been tested in the laboratory and in the clinic that a complete summary is beyond the scope of this essay. For further information of this sort the original papers of the following investigators might be consulted: Astwood, Bissell and Hughes (9), Astwood and VanderLaan (10), Bywater, McGinty and Jenesell (19), Chapman (23), Higgins and Larson (51),

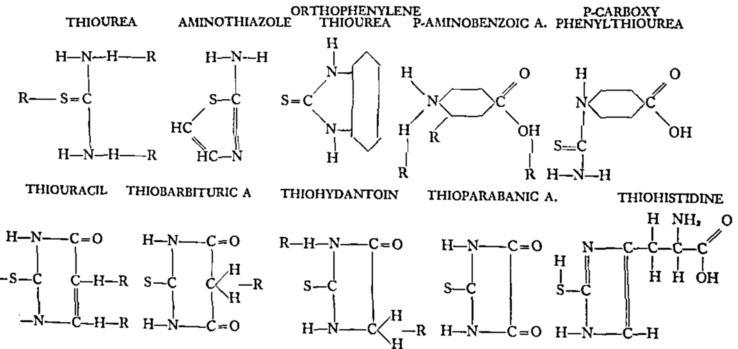


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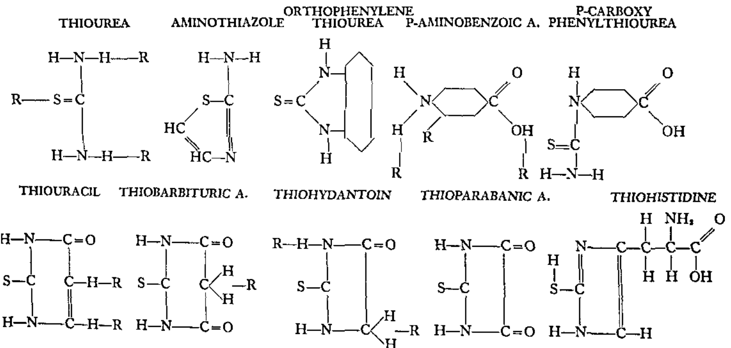


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less toxic. As a competitor thiourea itself might be considered, as suggested by Danowski, Man, and Winkler (27), (28). This compound, however, has the disadvantage of producing an unpleasant breath and taste in the mouth.

Other clinical studies, especially in Europe, have dealt with other chemical compounds which are more active than 6-*n*-propylthiouracil but may be more toxic. Notable among these is 6-methylthiouracil.

Many secondary problems have been exposed through the use of these agents, all of them tending to let the investigator probe more deeply into the inner secrets of thyroid metabolism. One of the first contributions of outstanding significance that the use of these drugs disclosed was the paradoxical divorce between structure and function. For decades pathologists in many clinics have undertaken to distinguish the hyperthyroid gland on morphologic grounds After the use of thiouracil, however, the marked goiter so produced usually is accompanied by a state approaching myxedema. In other words, despite the marked hypertrophy and hyperplasia which is so characteristic, such a gland fails to maintain even a normal output of thyroid hormone. The evidence for this categorical statement may be examined now. Much of the evidence was adduced by Astwood himself or has been described by him (8), so that only some of the most outstanding arguments will be discussed.

One of the most significant findings, subsequently confirmed in several laboratories, was the fact that in hypophysectomized rats no enlargement of the gland occurred. This finding indicated that under the action of thiouracil or its congeners, an increased secretion of pituitary thyrotropic hormone occurred. This increased supply of thyrotropic hormone then led to hypertrophy and hyperplasia of the target gland. If the goitrogen and thyroxine were administered

McGavack and Vogel (71), McGinty and Bywater (73), (72), Miller, Roblin and Astwood (77), Williams and Frame (115). Obviously, it will be some time before the final word is known concerning the most promising of these drugs from a clinical standpoint. At the present time, 6-normal propyl thiouracil would appear to lead the field. It is more effective than thiouracil and in therapeutic doses

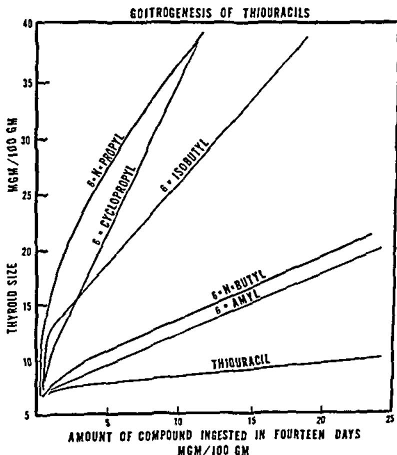


FIG 3. When thiouracil is substituted at the 6-position with various alkyl radicles, its activity as a goitrogen in rats is increased. From Williams, R. H, and G A Kay, *Am. J. M. Sc.*, 213:202, 1947.

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simultaneously to normal animals, no goiter appeared. In other words, if the pituitary were not goaded into hypersecretion by a general lack of circulating thyroid hormone, the presence of the goitrogen *in itself* would have no goitrogenic effect. This finding indicated clearly that the mechanism of goitrogenic action depended primarily on the failure of the gland to produce its normal daily quota of thyroxine or equivalent hormone. Furthermore, the goitrogen failed to prevent the toxic effect of large doses of thyroxine when administered to normal, thyroidectomized or hypophysectomized animals. In other words, no direct antagonism or neutralization occurred between the goitrogens and thyroxine.

These observations have localized the action of the goitrogenic agents to the thyroid gland itself It was noted in animals under the full influence of such drugs that the iodine in the gland very rapidly disappeared. Indeed, in young rats, the thyroid became nearly devoid of iodine in about a week. This loss of iodine was so rapid that young rats lost nearly half of the original store of iodine in a single day. This loss is truly phenomenal. Indeed, at this rate it will be seen that practically all of the thyroid reserve would be exhausted in about five days. Under clinical circumstances, however, particularly if the patient has previously received an excess of iodine in the form of medication, the follicular stores of human thyroglobulin may be so large that the patient will not want for thyroid hormone for several weeks. The resulting delay in decline of metabolism puzzled clinicians for a long time.

The physiological significance of such observations, however, is that the goitrogens do not impair the release of such hormone as is already stored in the gland. In short, the "blocking" process is concerned only with the manufacture of fresh hormone. Furthermore, the manufacture

of thyroid protein itself is not prevented, because it can be shown in chickens (8) that large collections of thyroid colloid can be amassed under the influence of a goitrogen. Such colloid, however, is devoid of iodine and has practically no endocrinological potency. These several findings *indicate, therefore, that the action of the goitrogen is related* to the synthesis of thyroxine and probably has its effect chiefly within the thyroid cell. The problem, therefore, resolves itself into the question of the successive stages of iodine metabolism within the follicular cell. In particular, the question concerns the various activities of those enzyme systems within the cell which are concerned with the manufacture of thyroxine. Inasmuch as at least two stages of this synthesis are recognized, as indicated in the preceding section, it is a matter of some interest to decide where the action of the goitrogens occur. Of course, it may occur at several stages and not at one alone. On the other hand, it may be possible that different goitrogens have different actions. This possibility is indicated by the experiments of Salter (101) who compared the action of thiouracil and thiocyanate in young adult rats. On analysis of the thyroids of these animals it was found that after thiouracil no diiodotyrosine could be formed and consequently no thyroxine. On the contrary, after thiocyanate little iodide accumulated, and practically no thyroxine; but there was a significantly disproportionate amount of diiodotyrosine present. These findings suggested that in the case of thiocyanate a different mechanism obtained than with thiouracil. Indeed, with thiocyanate there seemed to be a "bottleneck" after the formation of diiodotyrosine and preceding the formation of thyroxine.

As in so many other scientific problems, it is becoming apparent that quantitative aspects of this problem must be carefully considered. For example, McGinty (72) has

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two drugs. It was noted in adult rats that when the iodide intake was 135,000 micrograms daily and the potassium thiocyanate was given in doses of 324,000 micrograms daily, practically no iodide accumulated in the gland (98). In other words, the presence of thiocyanate had prevented the accumulation of iodide These experiments, of course, involve a very high intake of thiocyanate. On the other hand, diiodotyrosine was clearly present. Moreover, it is known clinically that under moderate doses of thiocyanate, a goitrogenic effect and the concomitant production of hypothyroidism can be avoided by administering simultaneously an excess of iodide Thus there is a sort of antagonistic effect between thiocyanate and iodide If iodide be present in excess, the gland will proceed to manufacture a sufficient quantity of thyroid hormone daily, even though it is not able to accumulate a large amount of iodide.

In contrast to this finding, was the observation that in the presence of thiouracil a very high concentration of iodide could be accumulated in the gland. This has been reported from other laboratories, and it is clear that thiouracil does not prevent the collection of iodide in the gland in concentrations considerably above that in the circulating body fluid. The ratios found under varying circumstances range from ten to several hundred. When extracts are made of such glands and the extracts subjected to heat coagulation, the iodine is recovered as inorganic iodide.

A large number of anti-thyroid substances belong in this second group, in which iodide in excess will not expedite the synthesis of hormone. These include the goitrogens which have been subjected to the most extensive clinical trial. That iodide has a definite effect upon the gland under these circumstances, however, has been recognized for some years by surgeons. When iodide is added to thiouracil in the pre-operative preparation the gland is firmer and less friable.

shown that the simple loss of iodine from the thyroid occurs more readily under the influence of a goitrogen than does hypertrophy and hyperplasia of the gland. In this finding Rawson (4) concurs. Moreover, it requires only three micrograms of racemic thyroxine daily to prevent the goitrogenic action of thiouracil in rats under certain conditions; whereas twenty to thirty micrograms daily are required to prevent loss of iodine from the gland. Furthermore, as Salter showed (98), there is a "hangover" of the effect on the gland which persists after the drug has been stopped. Likewise, McGinty has shown that when thiouracil has been given for a considerable time, doses as high as forty micrograms of thyroxine given simultaneously with thiouracil may fail to reduce the thyroid weight significantly or to restore the thyroid iodine to its normal value.

Moreover, the effect of these goitrogens is connected intimately with the level of iodine intake and the rate of iodine turnover. If, for example, a high iodine intake is given concurrently with thiouracil, the effect of the goitrogen is diminished. Furthermore, if the goitrogen is stopped, recovery from its action occurs more rapidly than on a low intake of iodide. At a very high intake of thiouracil, however, Salter (98) found that even a tremendous excess of iodine in the diet had little effect. Consequently, one can demonstrate different interrelationships between dosage of goitrogen and dosage of iodine depending upon the quantitative dosage of either substance. Indeed, at low levels of iodine intake, the absorption of iodine in the thyroid of the rat is partly prevented by thiouracil and related goitrogens. When, however, more iodine is given, a considerable concentration of iodine collects in the gland.

In comparing the action of thiouracil and thiocyanate in the presence of large doses of iodide, Salter (98) encountered a *distinct difference between the action of these*

system in the gland or in the follicular cells. It is assumed that such a peroxidase system might operate to change iodide into a state of higher oxidation and so lead to its combination with tyrosine. At the time of writing the interpretation

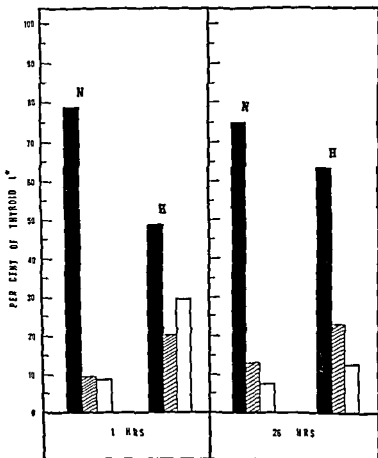


FIG. 4 Under the influence of thyrotopic hormone (indicated by H) the formation of thyroxine (hatched section) is expedited. Simultaneously the diiodotyrosine fraction (solid black section) is diminished Inorganic iodine is indicated by a clear bar. From Morton, M E, I. Perlman, and I. L. Chaikoff, *J. Biol. Chem.*, 140: 607, 1941.

Therefore, it may be removed more easily without hemorrhage or tearing. From the biochemical standpoint, however, it is by no means clear as yet how the iodide acts under these circumstances. It seems apparent, at least, that thiouracil does not interfere with the trapping of iodide. On the contrary, it prevents the formation of diiodotyrosine by combination of the iodide with tyrosine radicals Furthermore, it was shown by Chaikoff and his collaborators (80) that surviving slices of thyroid tissue bathed in Ringer's solution containing iodide were able to convert this iodide to organically-bound iodine. If, however, a goitrogen were added to the Ringer's solution, this conversion of iodide into organically-bound iodine failed to occur. Thus one is brought face to face with the most important problem in thyroid physiology at the present time, namely, how does the gland convert iodide into diiodotyrosine?

From what has already been said, it is clear that an oxidative process of some sort must take place in the gland which endows iodide with the status of elementary iodine or its equivalent Only by elevating iodine to a higher state of oxidation can it combine with the tyrosine radicals, displacing hydrogen atoms on the aromatic benzene nucleus Many years ago Blum (17) hypothecated an iodase system which would accomplish this work Modern investigations are now at the point of studying this phenomenon. As an analogue, Keston (61) studied the Schardinger enzyme in milk and found that this hypoxanthine oxidase was capable of converting iodide into organically-bound iodine. At the present time, no definite characterization of such an enzyme system in the thyroid gland has been made, although crude preparations of it probably have been prepared by Salter (96).

In cytohistological studies, Dempsey (29) has used differential stains in an attempt to demonstrate a peroxidase

even this drug is not entirely free from trouble. Of special interest is the use of thiourea supplemented by iodide as demonstrated by Danowski, Man and Winkler (27), (28). This synergistic effect of iodide in the presense of thiourea, given in relatively small doses, is puzzling in view of the facts encountered with the other goitrogens in the presence of iodide. Consequently the clinical findings deserve further investigation: because in the case of other goitrogens iodide apparently acts antagonistically to the goitrogen whereas in this particular instance, the goitrogen and the iodide appear to act synergistically. It is to be hoped that from the great activity which the goitrogens have aroused in various laboratories and clinics there will come a better understanding of the first stage of organic iodine synthesis within the gland.

Finally, there is also the physiologic possibility of blocking the action of the thyroid hormone in the peripheral tissues, irrespective of the activities of the gland. It was pointed out earlier in this section that attempts at producing an anti-hormone had been a failure from a practical standpoint. Nevertheless, the recent development of "phoney" vitamins, e.g., paramino-benzoic acid and pentoyl-taurine, has suggested that possibly a substance might be developed which would "jam" cellular enzymic machinery by competition. This general problem has been summarized by Woolley (119). In the case of the thyroid Woolley has suggested that compounds similar to thyroxine might interfere with the normal operation of enzymes in the peripheral tissues presumably by competitive blocking of those systems upon which the thyroxine acts normally. Woolley (119) actually has synthesized several new ethers of N-acetyl-diiodo-tyrosine. Some of these counteract the pharmacological effects of thyroxine as tested on tadpoles by the method of Gaddum (37) and by the aceto-nitrile test of Reid Hunt (55).

of these experiments is still under consideration, and attempts are being made to define the enzyme system in more precise terms.

As regards the further transformation of the organically-bound iodine to thyroxine, very little is known. Chaikoff (81) has shown that the presence of the thyrotropic hormone is important in furthering this reaction. His experiments are illustrated in Fig. 4. Furthermore, it has long been known that in the hypophysectomized animal the output of thyroxine falls even though the thyroid gland contains plenty of iodine. How much of this effect is primarily associated with the release of hormone as against active control of synthesis is impossible to say. It is conceivable that by expediting the release of hormone the synthesis of fresh hormone would be speeded up. Whether or not any of the goitrogenic agents act at this stage of the biosynthetic system has not been demonstrated.

It may not be amiss to add a word about the clinical status of these results. At the present moment, the routine use of the goitrogenic drugs in clinical medicine is limited to pre-operative surgical preparation. A number of clinics, however, are surveying the possibility of using these drugs as a substitute for operation, and are in the midst of observations to determine whether or not the mortality due to idiosyncrasy is higher than that of operation. A recent survey by Himsworth, indeed, indicates that the overall results from medical treatment compares favorably with those obtained under routine surgical treatment (53). In the case of thiouracil itself, a very considerable statistical information is now available which indicates complications in some twelve percent of cases. The most important complication is that of agranulocytosis in nearly two percent. The 6-normal propyl derivative tested by Astwood (11) was found to be much less likely to produce such complications. However,

inal thyronine structure. Some have fluorine instead of iodine, substituted on unusual carbon atoms, e g, 4' and 6'. In other compounds the alanine side-chain has been blocked by such substitutions as a benzoyl group. Among these compounds some have been found with weak activity resembling that of thyroxine. Among such compounds are 3, 5-diiodo-4 (3', 5'-diiodo-4'-hydroxyphenoxy) benzoic acid; 3, 5-diiodo-4-(4'-hydroxy-phenoxy) aniline; 3'-fluoro-3, 5 diiodo-*dl*-thyronine; 3', 5'-difluoro-3, 5- diiodo-*dl*-thyronine; 3'-fluoro-5'-iodo-3, 5-diiodo-*dl*-thyronine and 3', 5'-diiodo-4(4'-hydroxyphenoxy) 3, 5, diiodo hippuric acid. Moreover Cortell has reported that of seven thyroxine analogues which were examined for anti-thyroxine activity, one of them, 2', 6'-diiodothyronine, had the ability to prevent the inhibiting effect of both injected thyroxine and thyroglobulin on the hyperplasia of the rat's thyroid gland produced by treatment with thiouracil. However the compound was without effect on the untreated rat's thyroid in the doses used, and it showed no thyroxine-like activity when tested on thiouracil-treated rats

Such studies are still in the pioneer stage. They are of interest, however, on two counts. First, they demonstrate that when large dosages are employed, the specificity of chemical constitution is not as stringent as formerly supposed. Secondly, they suggest the bare possibility that ultimately chemical hyperthyroidism might be treated with an analogue of thyroxine which could block the effects of the hormone on general body tissues by competitive action. Possibly, also, such analogues might "deceive" the pituitary, and so suppress thyrotropic secretion.

Moreover, some of the compounds protected tadpoles against *the lethal effect of thyroxine. The two most active com*pounds studied were the p-nitro-phenyl-ethyl ether and the p-nitro-benzoyl-ether. The benzyl and butyl ethers were less effective. Curiously enough, the nitro-ethers had a weak thyroxine-like activity, as tested in tadpoles and mice, in addition to an anti-thyroxine property. This finding supports the view that a competitive action occurs which involves tissue enzymes. At the present time it is not known whether these compounds will be suitable for clinical therapy or not.

*Obviously, the demonstration of weak activity in ana*logues of thyroxine must be accepted as a reservation to or modification of *the statements already made concerning* the high specificity of thyroxine. These assertions hold true if a high potency of the drug is deemed essential. It is now clear, however, that characteristic therapeutic effects can be produced in human myxedema when large doses of certain chemical variants of thyroxine are tested. At a recent meeting of the American Goiter Association in Madison, Wisconsin, for example, the properties of tetrabromthyronine and tetrachlorthyronine were described by Lerman, Richards, Brady and Riggs. Seventeen molecules of the tetrabrom-compound were found equivalent to one molecule of thyroxine; whereas 250 molecules of the tetrachlor-compound were required. Nevertheless these compounds caused calorigenic effects with an accompanying fall in plasma cholesterol. Thus the myxedematous patients recovered from their hypothyroid state without a characteristic rise in the protein-bound iodine of the blood. These drugs also prevented the development of goiter in rats treated with thiouracil.

Similarly a number of compounds synthesized by Niemann have been tested both by Frieden and Winzler in California and Ruth Cortell in the East. These compounds exhibit a number of substitutions or modifications in the orig-

or sea food, or after the painting tincture of iodine upon the skin, perceptible rises in the circulating iodide occur. These are of little significance in themselves, but unless the *iodide is carefully separated from the protein-bound iodine* a false impression will be gained of the concentration of hormone available to body tissues. It was the merging of "hormonal" iodine with inorganic iodine which confused the picture in thyroid physiology for two decades. Indeed, several investigators had concluded that a study of iodine concentration in body fluids bore little if any relationship to thyroid activity.

Salter (94) has described four arbitrary levels of iodine intake which clarify the problem. These are termed, categorically, (a) the natural level, (b) the prophylactic level, (c) the anti-thyroid level and (d) the fibrolytic level. The approximate amount of iodine assimilated daily, corresponding to these categories, would be (a) 100 micrograms, (b) 500 micrograms, (c) 50 milligrams and (d) 1 gram. *Corresponding to each of these respective magnitudes of daily intake*, in general there is an increasing scale of fasting serum iodide concentrations. Thus the natural serum concentration might be about 0.5 microgram percent, the prophylactic level about three micrograms percent, the therapeutic level about sixty micrograms percent, and the fibrolytic level as high as 600 micrograms percent. In the last two instances the microanalyst must take special pains to eliminate the last trace of the iodide, which tends to adsorb upon protein precipitates and so to produce a spurious elevation of protein-bound iodine.

"Hormonal" iodine. The concentration of "hormonal" iodine in the circulating serum and body fluids seems to be higher for man than for most of the *common laboratory* animals. The human range of normal values usually is regarded arbitrarily as lying between *four and eight micro-*

CIRCULATING THYROID HORMONE

ANOTHER DEVELOPMENT in the chemical study of thyroidology has been an increased attention in recent years to the fate of the thyroid hormone in peripheral tissues and body fluids. Salter (96) has called attention to the metabolic circuit of the "thyroid hormone" in relation to iodine metabolism. He has pointed out that the iodide in the body is in constant circulation and is in approximate equilibrium throughout all body fluids. Travelling along with iodide in most instances is the "hormonal" iodine which contains the essential principle of the gland. Therefore, in careful studies of thyroid physiology, it is important always to specify at any given time and at any given point in the microcosm the concentration both of iodide and of "hormonal" iodine.

The circulating iodide. Under strictly physiological conditions the circulating iodide shows a very low concentration in body fluids, — usually under one microgram per cent. The problem has been greatly confused because this iodide concentration rises sharply when iodide medication is administered and indeed may reach values a thousandfold the natural value. In general, even in pharmacological concentrations, iodide seems to be distributed very much like chloride. The classic studies of Wallace and Brodie (113) showed that the ratio of chloride to iodide in most body water was the same at various points with the possible exception of the brain. Unfortunately, even in the absence of outspoken iodide medication, variations in the ingesta or in the environment may elevate the natural level of iodide several-fold. For example, after a meal of oysters

ganically-bound iodine resides in protein molecules which are readily capable of leaking through capillary walls. This may be a matter of some importance; because if the hormone is bound to protein in the circulating plasma it is difficult to understand how it might readily penetrate to the tissues, except by leakage of protein through capillaries.

This hypothesis has been tested partially by Salter (103) who has found that relatively little organic iodine is present in tissue fluids like the spinal fluid and pericardial fluid, which are poor in protein. To be sure, these fluids contain approximately the same amount of ionized iodine as any other body fluid, indicating that there is a ready equilibrium across capillary membranes. Furthermore, with the help of Doctor Donald Munro (102) it has been possible to show that the sodium salt of mono-iodo-methanesulfonic acid, when injected intrathecally, readily exhibits retrograde passage into the serum at high levels of concentration and within an hour or two. Therefore, the absence of protein-bound iodine in the spinal fluid and pericardial fluid constitutes an argument in favor of the hypothesis that the hormone travels in company with proteins There is still some question as to the exact nature of the binding. At the moment it seems most likely that a direct peptide linkage exists, inasmuch as thyroxine is an amino acid and could be incorporated readily into a long peptide chain to form a protein molecule. The experiments of Shoenheimer and his school (106) on the lability of the protein chain and the continuous interchange of amino acids therein would seem to favor this point of view Nevertheless, it still remains possible that the hormone travels along with certain protein fractions in consequence of accessory valences or coordinate bonds The author does not favor this last hypothesis but further proof is needed.

In any case, one would expect to find in lymph, e g., in *the cervical lymph, less protein than in the* plasma but a

grams percent. As ordinarily determined, this chemical fraction is separated from the serum by precipitation of the serum protein; consequently some investigators prefer to call "hormonal" iodine "plasma-precipitable" iodine or "protein-bound" iodine. There is now a considerable body of evidence which indicates that these terms, although chemically distinctive, actually measure the same thing from the physiological standpoint The author prefers the term "hormonal" iodine because it recognizes the physiological validity of certain physico-chemical procedures which in themselves are only a means to an end.

When thyroxine is injected into the blood stream it very soon becomes incorporated into the serum protein. Likewise, the natural hormone is found associated with characteristic fractions of the plasma protein. The evidence for this statement is now good. A number of years ago Salter and Bassett (97) precipitated *seriatim* the successive fractions of horse serum and of human serum. They showed there was a preferential distribution of the iodine therein (12). Relatively little organic iodine was found in the fibrinogen and the largest (gamma) globulin molecules, and very little in the smallest albumin molecules Somewhere near the dividing line between the albumin and globulin protein fractions, however, samples were encountered which showed high concentrations of organically-bound iodine. In the earliest fractions, which were only crudely separated, these materials seemed to be largely in the lowest albumin fraction Recently, in work with very pure fractions supplied by Professor Edwin J. Cohn, even higher concentrations have been found in the smaller globulin molecules. There are a number of physio-chemical difficulties connected with the purification of these fractions and only a preliminary statement can be made at this time From a physiologic standpoint, however, it would appear that the or-

of the same order as that of the blood. They found that it was considerably higher in the tissues of mice than of the steer. Recently, Salter has made measurements of the iodine in the skeletal muscles of rats. It appears (95) that rat muscle contains both inorganic and "hormonal" iodine just as does the plasma. Investigations are now in process to fractionate the muscle protein in somewhat the same fashion as the plasma proteins have been separated. The working hypothesis, by which this investigation is justified, is that when thyroid hormone enters peripheral cells it unites with colloidal constituents of the cell sap to form a sort of superenzyme. This combination might perhaps be called "thyrenzyme."

It has long been known that enzyme systems like the amylase of liver and the Warburg-Keilin cytochrome oxidase systems show increased activity in the hyperthyroid animal. Until the isolation of the organically-bound iodine in muscle has been accomplished, the mechanism of the action of the thyroid in the peripheral tissues will not be understood.

Roughly speaking, the order of magnitude of this peripheral iodide is about ten micrograms percent when referred to fresh wet muscle. Such concentrations require the development of new techniques It will be recalled that the concentration of circulating hormone when expressed as a negative logarithm is about — 7. In other words the concentration resembles that of the hydrogen ion, of digitoxin in therapeutic doses, of thiamine, and of the estrogens and androgens. Probably a new field of clinical catalytic chemistry must be developed by clinical investigators and similar procedures must also be extended to animal work. Because, in general, laboratory animals are smaller than men, the clinical catalytic chemists have been doing some of the advanced work in this field. It will be noted that as compared with glucose the physiological concentrations of these

relatively higher concentration of iodine with respect to protein. This would be expected because of a preferential leakage of smaller protein molecules which are rich in iodine content. This is indeed the case as found by Salter (96) in experiments aided by Doctor Cecil Drinker (32). It still remains a problem how the hormone reaches the interior sap of cells from the bathing fluid. However, this question lies within the realm of cell physiology and may be a long time in the answering. For the moment, it is at least of interest that one can trace the protein-bound iodine into the blood and into the lymphatics which drain the tissue spaces. The assumption, therefore, is reasonable that the tissue spaces surrounding the cells contain a medium of protein-bound iodine which leaves the tissues and constitutes (as it were) a reservoir for the hormone. In this sense, the plasma proteins might be considered as a carrier of thyroid hormone in somewhat the same sense that oxygen is carried by the hemoglobulin of the red cells. The analogy, however, is only crude and must not be pursued too closely.

In consideration of the nice equilibrium which is maintained between the cells and the thyroid gland, and in view of the very potent effect which an excess of thyroxine may produce, perhaps it is fortunate that this highly active hormone is held in check by combination with large colloidal molecules. One hesitates to think what might happen to hydrolytic and oxidative enzyme systems in the cells if an excess of the hormone were let loose without restraint into the cell sap. It may not be too fantastic to imagine that a state resembling spontaneous combustion might result!

The next problem, obviously, is the fate of the thyroid hormone in tissues. Investigations of this problem are still in their infancy, but a little evidence is available. For example, McClendon and Foster (70) reported that the iodine content of peripheral tissues like muscle was approximately

is to be done systematically, the necessity for ultra-microchemical procedures for determining total iodine becomes all the more pressing.

An interesting development along such lines has been the application of a reaction which Sandell and Kolthoff (104) studied some years ago, i.e., the reduction of ceric ions to cerous ions. These investigators demonstrated that the ceric salts are reduced very slowly by arsenious acid unless the reaction be catalyzed by certain substances, among which iodide ions are pre-eminent. Because the ceric ion is yellow and the cerous ion colorless, the rate of the bleaching of a known concentration of ceric ion is a convenient way of measuring the rate of reaction. This method was suggested by Chaney (21) as a means of studying the plasma iodine. The method has been extended by Salter and McKay (100) for use in tissues as well as blood serum. It is possible by this method to measure satisfactorily the "hormonal" iodine in 1 milliliter of plasma or in 250 milligrams of muscle. The biological material is first ashed in alkaline medium and then allowed to react with ceric ions and arsenious acid in an acid medium.

It turns out that the rate of reaction under certain specified conditions is directly proportional to the concentration of iodide. The reaction is calibrated against known concentrations of iodide and a linear curve is drawn which may be applied in determining the concentration of unknown samples The equation follows:

$$-\frac{d\left[Ce^{++++}\right]}{dt}=k\left[I^{-}\right]$$, for the reaction

$$2Ce^{++++}+H_3AsO_3^{-}+H_2O=2Ce^{+++}+H_3AsO_4^{-}+2H^{+}$$
(Yellow) (Colorless)

By this method it is possible to determine one-ten millionth

catalysts are over 1000-fold smaller; this difference is reasonable because substances like glucose are the substrates upon which these catalysts operate.

In the fractionation of muscle, as in the fractionation of plasma proteins, one must take pains to separate the (inorganic) iodide from the protein-bound material. If iodide medication has been administered previously in large dosage, this is no easy task: because the iodide ions tend to adsorb upon the muscle protein and so give a false impression of organically-bound iodine. In the case of plasma, an analogous situation has been demonstrated by Riggs and his collaborators (90); because in the presence of very high circulating iodide in Graves' disease under treatment, the hormonal iodine actually falls as thyroid secretion is inhibited. In order to demonstrate this fact, however, the chemist must take special precautions to dialyze away or wash out the large excess of iodide ions which tend to swamp completely the true "hormonal" iodine. It is very easy to attain spuriously high values under such circumstances, and such values are entirely misleading.

Danowski has shown that after many days or weeks of treatment with large doses of potassium iodide, the blood serum of normal men may contain over 25 micrograms per cent of protein-precipitable iodine

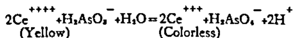
In order to measure such concentrations of catalytic magnitude, newer methods must be developed. In part, the use of radio-iodine is an answer to this problem and much will be said about this method in the next section It must be realized, however, that radio-iodide gives only absolute concentrations and is of greatest value when the total iodine (i.e., stable iodine plus radio-iodine) is determined at the same time. In other words, it usually is more important to determine the "specific radio-activity" of a tissue than to determine the concentration of radio-iodine alone. If this

is to be done systematically, the necessity for ultra-microchemical procedures for determining total iodine becomes all the more pressing.

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By this method it is possible to determine one-ten millionth

of a gram of iodine within an accuracy of five per cent, after one has had sufficient experience with the procedure. When combined with a preliminary chemical separation or when combined with simultaneous determinations of radioactivity this method can supply very valuable data in small animals as well as in men and children.

It is indeed highly gratifying that the determinations of circulating serum or plasma iodine by various methods in various laboratories now yield results which are in substantial agreement. This is true of data reported from various parts of the United States and indeed from the several distinct sections of the world. The serum "hormonal" iodine is practically non-existent in advanced myxedema and may reach values over twenty micrograms percent in thyroid storm The term "thyroid storm," however, is not to be used as a direct indication of high values of circulating hormone. It is merely a condition of collapse which may occur in various individuals at various levels of thyroid activity. Likewise, high serum iodide and "hormonal" iodine may not necessarily indicate spontaneous thyroid activity. For example, when large masses of muscle are broken down by trauma, the iodine content thereof is liberated. The result is an abnormally high iodide in the serum and perhaps a slight elevation of circulating hormone. Even more striking is the liberation of both iodide and hormone when destructive agents are allowed to operate upon the thyroid gland itself. For example, the use of radio-iodide as discussed in the next chapter may produce rapid destruction of thyroid tissue. Under these circumstances serum iodide may occasionally reach 100 micrograms percent and the "hormonal" iodine may approach values of twenty-five micrograms percent. This is especially true if widespread thyroid metastases are present throughout a patient's body,

undergoing destruction simultaneously as result of the trapping therein of radio-iodine.

Hormone of extrathyroidal origin. Two laboratories have contributed evidence that in non-thyroidal tissues there may reside an ability to form thyroxine or its equivalent. This evidence is of two sorts. The data of Chapman (22) from rats which had been thyroidectomized have suggested that the behavior of these animals with reference to salt and water balance showed a residual capacity to produce thyroid hormone. The data of Morton and Chaikoff (79) (80) were based on the isolation of radio-active material which behaved like thyroxine in that the radio-iodine remained with known a thyroxine carrier on repeated crystallization. Both of these investigators have concluded from their data that the tissues at large retain a residual capacity for manufacturing hormone. This finding is of some interest from the standpoint of biochemical evolution. Nevertheless, two comments are necessary. In the first place, it is not altogether certain that the phenomenon may not be an artifact. For example, in view of the experiments of Block (16) and of Harington (43) that thyroxine can arise from pure diiodotyrosine *in vitro,* it is conceivable that during the course of the manipulation in the chemical laboratory some thyroxine was formed. Even granting that the finding is *bona fide,* it still must be noted that the amount of extra-thyroidal hormone so formed is extremely small. It might suffice perhaps to carry on metabolism at the level of a fungus or lower form of life. It seems quite clear, however, that if a mammal is thyroidectomized completely, its tissues in general lose the capacity to maintain a metabolic turnover at any considerable speed and eventually the entire economy of the organism becomes deranged

This process takes time, to be sure. Indeed, as point...

out by Magnus-Levy (18) nearly fifty years ago, the thyroidectomized organism shows a semi-logarithmic rate of calorigenic decline as the inherent hormone in the tissues becomes exhausted. As will be pointed out in the next section, there is some evidence in the lower forms of life that iodide may be fixed preferentially by certain organs which are not yet as recognizable as actual thyroid tissue. Interesting as it is to think of the chemical evolution of thyroid synthesis and a possible atavistic remnant of this function in all tissues, the problem would seem to be not of primary quantitative importance in the metabolism of an actively functioning mammal. As yet, it remains undetermined whether diiodotyrosine exists in peripheral tissues and, if so, in what proportions to the alleged natural hormone.

Riggs and his collaborators (90) have studied the hormone in the circulating plasma of hyperthyroid individuals when treated with a heavy dosage of iodide. Under these circumstances secretion from the gland into the blood stream is temporarily blocked Consequently, even though the iodide in the plasma mounts sky-high, the true "hormonal" iodine falls Indeed, it seems to fall a short period, i e., twenty-four to forty-eight hours, ahead of the fall in the basal metabolic rate Although tissue iodine studies have not been made under such circumstances, it seems reasonable to assume that the plasma hormone is declining faster than is the tissue hormone, which presumably is petering out by a natural decay process Therefore, although ordinarily one thinks of the serum "hormonal" iodine as being in equilibrium with tissue iodine throughout the body, there are conditions of metabolic change in which this need not be so.

The incorporation of iodine in human serum proteins, as described by Danowski after prolonged treatment with heavy dosage of potassium iodide, is a case in point.

In addition to the instance just cited there is a converse

one in which a large dose of thyroid hormone is administered from an exogenous source. In particular, if thyroxine be injected intravenously, for a short interval there is a very high circulating "hormonal" iodine. Within a matter of a very few hours, however, it is impossible to separate the injected thyroxine from the plasma protein. The assumption is justified that the administered thyroxine has been incorporated into the circulating protein and, therefore, that it is truly protein-bound. Winkler and his colleagues (117), (118) have shown that under these artificial circumstances the patient's own thyroid may exert a "detoxifying" effect. In short, there seems to be another function of the gland besides the manufacture and storage of hormone This is to protect the organism against an excess of hormone. This protection consists of at least two features. The first of these processes is the cessation of natural secretion by the gland. The mechanism involved presumably is suppression of pituitary thyrotropic activity, with the result that the gland assumes the so-called resting state as viewed microscopically. The other feature of this protection of the organism from an excess of exogenous thyroid would appear to be a direct destruction of the extraneous hormone by the gland The chief evidence for this is that individuals with normal thyroids show a rather variable but high resistance to metabolic change when given an excess of thyroid medication On the contrary, truly myxedematous, i.e., athyreotic, individuals behave in a much more uniform fashion towards the exogenous administration of thyroxine. In fact, they are known to be much more responsive or sensitive to thyroid medication In this sense the gland may be thought of as a buffer or tampon which serves to protect the organism against overenthusiastic treatment by the physician. It is not clear how such a protective mechanism was developed in the course of evolution. Presumably it is purely accidental, but the effect

is the more puzzling because there is no evidence available as yet that any organic iodine compound can penetrate the gland. Indeed, what little evidence there is (34) suggests that diiodotyrosine and other organic compounds containing iodine can contribute to the thyroid's stores only after they have been decomposed. Thereupon the iodide released by decomposition can enter the follicular cell just as would iodide from any nondescript source.

APPLICATIONS OF RADIO-IODINE

AFTER THE pioneer experiments of Hevesy (50) in the use of radio-active indicators in physiology, great progress could not be made because of the lack of suitable isotopes for various types of physiologic work. In 1934, however, Joliot and Curie (58) started a new field in biophysics by showing that new kinds of radio-elements could be produced artificially (26). Since that time the number of isotopes of iodine has increased steadily so that now in addition to the stable isotope I^{127} there are some thirteen others known. Of these those that have been used most are the element with a half-life of twenty-five minutes, I^{128}; the thirteen-hour isotope, I^{130}; and the eight-day isotope, I^{131}. Moreover, the specific radio-activity of these materials has been increasing steadily so that in the case of the isotope last named, experimental effects have been followed for as long as three months in some instances.

In the earlier work with radio-iodine, the specific radio-activity of the material available was low. For this reason, it was necessary often to use considerable amounts of inert material as its carrier, in order to attain a sufficient concentration of radio-active isotope to be measurable. This analytical difficulty was unfortunate in some instances because it meant that the experiment was not a purely physiological one. In short, it was merely a study of the effects of large doses of iodide upon the thyroid gland. Inasmuch as large doses of iodide are known to influence the activity of the gland, such experiments were not valid in studying normal thyroid physiology. Nevertheless, the investigators often

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unwittingly interpreted their results as having a physiologic rather than a pharmacologic significance.

In recent years, however, the radio-activity of the available isotopes has steadily increased so that this problem of too much carrier is rapidly disappearing. In its place a new problem is arising, namely, the question whether the specific activity of the material used may be so strong as to cause injury to the cells which are being investigated. If this seems a fantastic thought, one has only to consider that radio-iodide is now under trial as a therapeutic agent for its gross destructive effect upon the thyroid. More will be said later concerning this destructive effect.

The advent of the radio-tracer techniques has been hailed as the discovery of the most important biological tool since the microscope. Nevertheless, it must be admitted at the present time that, as one surveys the large amount of data which already has been amassed by this method in connection with thyroid problems, the great bulk of it merely confirms data which had long since been accumulated by straightforward chemical analyses. In other words, if the radio-tracer technique is to justify itself as an advance in the study of thyroid physiology, eventually it must yield new biological concepts. There is every promise that it will do so, when the investigators who use it are fully aware of its applications and implications. Too much literature, however, has already accumulated which is merely confirmatory of pre-existing well established data.

For example, in the blood plasma of the normal rat, the concentration of "hormonal" iodine, H_o, remains steady at about 2.5 micrograms per cent. If the investigator wishes to apply radio-iodide tracer technique to estimate the turnover rate of the thyroid hormone in the blood, he must study simultaneously stable iodine and tracer iodine in both the organic and inorganic forms. This is true because the con-

centration of hormone constitutes a dynamic equilibrium Whenever a little fresh radioactive hormone appears in the blood stream, part of it is soon metabolized together with some previously existing hormone. Accordingly, the rate at which the hormone turns over can be estimated from the following equation:

$$\frac{dH}{dt}\left(1-\frac{L}{H_o}\right)=\frac{dL}{dt},$$

In this expression H indicates freshly formed hormone and L labelled hormone which remains unutilized. At any given instant a definitive value for L can be computed from experimental measurements of A, the observed radioactivity, and from σ the specific radioactivity of the inorganic iodine in the plasma. The author has found experimentally that in the normal rat, once injected with tracer iodide, these values change in accordance with the following relationships:

$$\frac{-d \log \sigma}{dt}=k,$$

$$\frac{dL}{dt}=\frac{dA}{\sigma dt}$$

When the investigator has evaluated L for an appropriate time interval, the amount of fresh hormone which has accumulated in that time can be calculated from the following equation:

$$H_{t_o}=-2.5\ Ln\left(1-\frac{L_{t_o}}{2.5}\right)$$

During the past decade much work has been published which cannot be interpreted physiologically because the experimental data were limited to measurements of radioactivity

The thyroid lies so superficial to other structures in the neck that radio-activity from a tracer can be detected by an outside pick-up. Under such circumstances, the subject may be asked to swallow a glass of water containing a little radio-iodide and the monitor in the amplifying machine turned on. For a brief time very little happens except for the occasional click of a cosmic ray coming in from outer space. In addition, there may be certain other local "background disturbances." After about five minutes, however, a very loud and increasing barrage of discharges can be heard which announces the arrival of the first tracer atoms

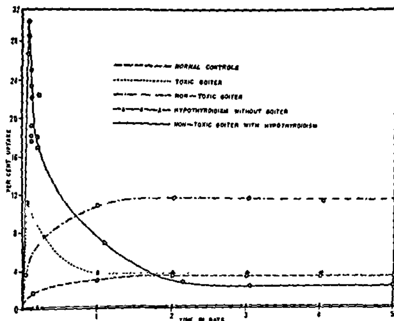


FIG. 5. When a single dose of labeled iodine (14 mg) is fed to each of several individuals with typical thyroid disturbances, characteristic measurements of labeled iodine can be obtained *in vivo* by applying a Geiger counter to the neck, anteriorly Modified from Hamilton, J. G, and M. H Soley, *Am J. Physiol.*, 131-139, 1940-41.

of iodine within the thyroid. In about fifteen or twenty minutes, and certainly within an hour, this barrage nears its maximum volume: indicating that the concentration of iodide trapped within the gland is now approximately constant.

As shown in the experiments of Hamilton and Soley (40), indicated in Fig. 5, such results yield quite comprehensible results in classical cases. For example, the myxedematous patient who is an athyreotic, shows no collection of radio-tracer. The normal patient shows a definite, but not a spectacular, amount. The hyperplastic goitrous gland, on the other hand, because of its large mass of parenchymatous tissue traps a considerably greater amount than the normal. Finally, the empty follicles of the gland in Graves' disease fill up at a surprising rate and then proceed to pay out the iodine as new hormone is manufactured. Consequently, in brisk hyperthyroidism there is a very high influx of iodine at the start and later an efflux of iodine, as shown in the figure. Such experimental procedures are spectacular and make good demonstrations. They do not, however, always advance our understanding of thyroid physiology; which, after all, is the chief objective in the use of radio-tracer substances as a biological method In other words, more thought must be given to pre-existing knowledge and in the careful planning of experiments if this procedure is to realize the great expectations which were once held for it. Nevertheless, already some very definite advances have been made, and it is the purpose of this essay to point out a few of them.

Radio-autography. One very important advance, made through the use of these tracers, is that it is now possible to identify tracer elements anatomically so that the metabolism of such materials can be localized even to a single layer of cells Some of the original work of this sort, performed by Hamilton, Soley and Eichorn (41) is illustrated

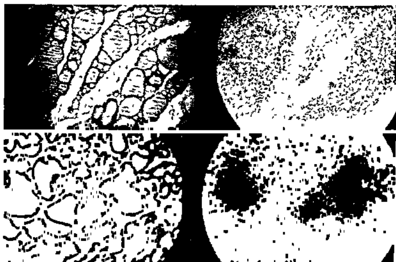


FIG 6. Radio-autographs (on the right) of normal thyroid tissue (above) and hyperplastic tissue (below) In each case a routine stained histological section is shown at the left. From Hamilton, J G The Use of Radioactive Tracers in Biology and Medicine *Radiology,* 39 557-558, 1942

in Fig 6 It may be remarked that the parathyroid gland, featured in the ordinary hematoxylin-eosine section as a control, does not take its own picture on a radio-autographic film. The reason for this is obvious, namely, that it does not trap radio-iodine In contrast with the normal thyroid, the thyroid of a goiter which is filled with distended cysts shows a very sparce accumulation of iodine What is more important, the large cysts apparently have no traffic with the newly acquired iodine This is the best demonstration to date of the inactivity of the contents of these large cysts In cancer, it is possible to compare the activity of normal tissue with malignant tissue It was observed that the normal tissue shown in Fig 6 trapped iodine and took its own photograph in consequence On the contrary, often malignant tissue took up very little radio-iodine This find-

ing is in harmony with the clinical observation that hyperthyroidism only rarely results from malignant degeneration of the thyroid. As will be pointed out later, however, certain metastases of thyroid tumors have shown a high avidity for iodine and this property has been utilized in an attempt to exterminate them by heavy radiation (35).

This tracer technique has been applied to the embryology of the thyroid, and in particular to the evolution of its capacity to trap iodine. For example, Gorbman (39) found that iodide could be trapped specifically in cyclostomes and higher vertebrates. In studies of the "endostyle" of such lower forms, it was possible to demonstrate a single epithelial layer which could concentrate administered radio-iodide. The analogous organ in the sea-squirts, on the contrary, shows no such property even though they already possess a primitive pituitary anlage. As Goldsmith (38) has pointed out, in lower forms such as Amphioxus no such property exists. Nevertheless, at the time that this single layer of cells in cyclostomes is able to trap iodide, it is difficult to recognize the tissue as a true thyroid anlage. It had better be described simply as a hypobranchial groove or stolonic septum derived from the ventral pharynx Of course, we are speaking here of the development of the thyroid gland *per se.* The fixation of iodine in the skeleton of sponges and sea-fans has been known for a long time What it does no one knows, except that it forms diiodotyrosine

Another application of the use of iodide, which is promising but as yet not well tried, is the use of the principle of "isotope dilution" as a method of determining the concentration of naturally occurring iodine. This method is based upon the determination of the specific radio-activity of a certain sample, even though the recovery is not complete. For example, a known amount of inert iodide is added to a radio-active sample Then, after certain unknown losses

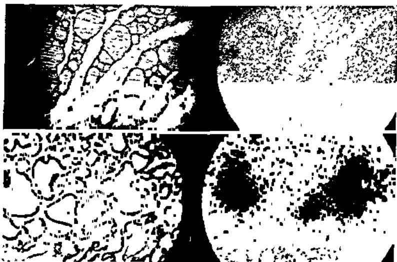


FIG 6. Radio-autographs (on the right) of normal thyroid tissue (above) and hyperplastic tissue (below) In each case a routine stained histological section is shown at the left From Hamilton, J G The Use of Radioactive Tracers in Biology and Medicine *Radiology,* 39 557-558, 1942

in Fig. 6 It may be remarked that the parathyroid gland, featured in the ordinary hematoxylin-eosine section as a control, does not take its own picture on a radio-autographic film. The reason for this is obvious, namely, that it does not trap radio-iodine In contrast with the normal thyroid, the thyroid of a goiter which is filled with distended cysts shows a very sparce accumulation of iodine What is more important, the large cysts apparently have no traffic with the newly acquired iodine This is the best demonstration to date of the inactivity of the contents of these large cysts In cancer, it is possible to compare the activity of normal tissue with malignant tissue It was observed that the normal tissue *shown in Fig 6 trapped iodine and took its* own photograph in consequence On the contrary, often malignant tissue took up very little radio-iodine This find-

ical fraction containing iodine, he can record the rate of change which is going on in each of those fractions from their respective specific activities. This problem has been well applied by Leblond (63) in the study of the several biosynthetic steps which iodine follows in its course from iodide to thyroxine within the gland. Few other investigators, however, have utilized this procedure as yet. Consequently, although information is rapidly accumulating with respect to normal animals as well as in various clinical disturbances of man, many of these results must be regarded as empirical. Because often one has no knowledge of the actual chemical fractions already present in the gland when the tracer dose is administered, it is unfortunately true that many of these data will never be susceptible of mechanistic interpretation. More and more stress, therefore, must be laid upon the combination of radio-tracer measurements with classical microchemical determinations, so that the experiment may have a true physiologic significance. For this reason it becomes rather difficult to present many of the results now in the literature in a coherent sequence. They must be regarded as suggestive data which exhibit certain correlations. One must be content for the nonce to indicate the observations as such, without hope of weaving them into a general mechanistic interpretation of the action of the gland.

Another difficulty which may supervene to plague the investigator is the exchange or atomic interchange reaction. The danger of this technical complication was pointed out by Schoenheimer (106) in his original work on the "metabolic pool." It has been neglected, however, by many investigators in the thyroid field. Miller, however, pointed out that an interchange reaction could occur under certain circumstances (76). Salter and Peacock (95) followed this up and found that in microchemical work as much as seventy per-

the recovery of the radio-iodide is determined again. Thereupon the same correction factor for loss can be applied to the whole sample as found for the radio-iodide or *vice versa.* This method has been suggested as a means of determining the concentration of serum "hormonal" iodine and the concentration of hormone in other tissues. As yet, however, it is on trial.

An important principle in the use of these tracer substances which was originally expounded by Hevesy (50) has been neglected by many investigators in recent years. This is the principle of determining the specific radio-activity of iodine in tissues, namely, the ratio of the radio-active material to the total amount of iodine present. It has been the custom among many investigators simply to measure radioactivity In the occasional case of an empty thyroid gland, as in brisk Graves' disease, the simple determination of radio-activity may be adequate, because all of the iodine collected by the gland has the same specific activity as the carrier which was administered to the organism. This assumption, however, is far from justifiable when truly tracer dosages are used. For example, it is now the custom for investigators to administer to an organism as large as a man a fraction of a microgram of total iodine. The specific activity of this tracer dose, however, is so high that its relatively few million molecules can be detected readily in the gland after the material has been distributed through the body fluids

The interpretation of such experiments, however, becomes exceedingly difficult. Under these circumstances the tracer is acting merely as a sort of indicator, showing the general trend of metabolism within the gland As it becomes mixed indiscriminately in all the metabolic pathways which any iodide atom might traverse, the observed concentrations will represent a kinetic trend. If the investigator, therefore, takes the trouble to analyze the total amount of each chem-

It remains an interesting finding which at some future date may have important developments.

There remain to be described some interesting physiologic and clinical observations which have been made with the tracer technique. For example, Schachner, Franklin and Chaikoff (105) studied the fate of radio-iodine in surviving slices of the thyroid gland obtained from the dog, sheep and rat. These slices were placed in a bicarbonate-Ringer's solution containing under one-tenth of a microgram of stable I^{127}. To this solution were added tracer amounts of I^{131} too small for chemical measurement. The slices of gland were incubated and equilibrated with the Ringer medium. After two hours the distribution of the tracer among the inorganic, diiodotyrosine and thyroxine fractions (respectively) was determined. The latter ratio was roughly six to one. These investigators, furthermore, fractionated the tissue and separated the "D" and the "T" forms after an admixture of a crystalline carrier consisting, respectively, of diiodotyrosine or of thyroxine. They found, using over 300 milligrams of rat's thyroid gland, that in three hours as much as twelve percent of the tracer was incorporated into the thyroxine-like fraction and seventy percent into the diiodotyrosine-like fractions. Control experiments in which the slices were first homogenized to form a "brei" failed to show any such transformation. This ultra-microchemical method is of considerable interest as a possibility of studying biosynthesis in surviving tissues.

As mentioned earlier, Chaikoff's group (80) have also studied the formation of the diiodotyrosine-like and thyroxine-like fractions in completely thyroidectomized rats. In the animals employed, the rate of oxygen consumption had been reduced considerably as the animals approached a marked hypothyroid state. At this stage radio-iodide was in-

cent of free radio-iodide ion might interchange with a like amount of inert diiodotyrosine: thus giving the false impression that diiodotyrosine had been formed *de novo* in the material under study. Particularly influential in this respect was the high acidity of the solution. At pH 2, the interchange was complete in less than forty minutes. Doubtless other variables would influence this reaction, e.g., temperature. There is some hope that this complication can be avoided by the use of chemical fractionation methods which remain within the neutral or alkaline range of acidity. Nevertheless, this interchange represents a hazard which must be borne in mind especially by those who would investigate the intermediary metabolism of the gland.

An interesting anomaly was presented by Corson, MacKenzie and Segrè (25) when they studied the effect of "astatine" or element 85. There is some discussion among the nucleophysicists at the present time as to its proper name. It was prepared first at the Radiation Laboratory at Berkeley, California. It was found to have a half-life of about seven and one-half hours. It was prepared by bombarding metallic bismuth with alpha particles. Although it was expected that the material would be a halogen, it had so many properties resembling those of metals that at first there was some doubt as to its identity It was interesting that part of the evidence adduced in favor of considering it a halogen was the ability of the guinea-pig's thyroid to trap it preferentially. The thyroid of the guinea-pig is not as active in picking up this element as with iodine; nevertheless, the storage of element 85 is still of the same order as that for iodine. In moderate doses, indeed, as much as ten percent of the administered element may be trapped in the thyroid. At the present writing no further application of this phenomenon has been made to physiology or therapy.

nine cases of hyperthyroidism which were treated mainly by such a procedure of internal radiation. For example, in patients with goiters weighing sixty to seventy-five grams, as verified at operation, they found that doses of five to twenty-five millicuries of radio-iodide were highly effective in controlling the disease. In clinical terms, about eighty percent of these cases were highly successful. Much the same experience had been obtained in California by Soley (107), with the early observation in 1944 that occasional cases of permanent myxedema were produced In the absence of Hertz during the war, the series in Massachusetts was continued by Chapman and Evans (24). More than twenty-two additional patients with hyperthyroidism were studied in which the only therapy used was radio-active iodide. Between fourteen and seventy-nine millicuries of twelve-day iodine were given to each patient supplemented by about one-tenth of the amount of eight-day iodine. The carrier was one-half a milligram of iodide in the form of sodium iodide. The material emits beta rays in the thyroid, which have a minimum scatter range of only a few millimeters of tissue. Therefore, the surrounding structures in the neck are not involved, as used to be the case when heavy x-ray therapy was given from an external machine.

The actual dose per patient of about fifteen millicuries of twelve-hour iodide, carried in about one milligram of ordinary iodide, could produce a total radiation in the thyroid tissue of some 4000 roentgens, effective over the course of at least a fortnight. It is interesting that about one-fourth of the patients treated in his manner showed the so-called "roentgen ray sickness." Furthermore, about one-sixth of the patients developed myxedema after therapy. In general, it is apparent that this method of therapy needs to be investigated further before it can be evaluated permanently. At the moment it may be said that it offers one more method

jected. Four days later it was found that a third of the radio-iodine present in the liver and small intestine was organically-bound. When these organs were subjected to chemical fractionation, about one-fifth of the tracer iodine was in the "D" form and about one-twelfth in the "T" form. When this last fraction was subjected to repeated crystallization with pure thyroxine, the radio-iodine remained in the thyroxine at a constant radio-activity. This result is interpreted as showing that the radio-iodine in the liver and intestine had actually been transformed into thyroxine. Several other important approaches of this sort are being pursued in various laboratories which ultimately may yield important information with respect to the localization of the thyroid hormone in various tissues. They may also give important evidence concerning the mechanism of its synthesis at various chemical stages in the gland itself.

Therapy with radio-iodide. In 1940 the author in summarizing the endocrine role of iodine mentioned the possibility of radiation therapy with radio-active iodine (94). Indeed, Hertz, Roberts, Means and Evans (49) had made a preliminary calculation of the dosage of such material which would be needed for effective therapy. They based their calculation on the assumption that a dose of 100 R units within the thyroid would be desirable. They assumed that normal tissues could tolerate safely one-tenth of an R unit a day for a considerable period. On the basis of experiments with rabbits, they assumed that a patient with 75 grams of thyroid tissue could be given about 750 millicuries of radiation on such a basis. At that time, a dose of this magnitude was just within possibility: therefore, it might be possible for about an hour to have within the thyroid itself the equivalent of a gram of radium.

At the present writing this dream has already been fulfilled. Hertz and Roberts (48) studied a series of twenty-

high values. Indeed, the patient may approach a state of "thyroid storm." On analyzing the blood from such a patient, the author at first thought that it must be contaminated because it contained so much free iodide. In addition, however, it contained a high concentration of "hormonal" iodine. In this particular case, although a very high dose of radiant energy had been administered, the weight of total iodide *was a fraction of a microgram. This tiny amount could not* possibly have accounted for the high concentration of circulating iodide in this case. It must be concluded, therefore, that when massive destruction of the thyroid gland occurs, iodine can be poured into the blood stream in excess. This iodine may be in the form of iodide, and consequently may do no great harm. It may also exist partly in the form of hormone, in which case the patient is subjected to a brisk and sudden bout of hyperthyroidism.

To what extent various malignant tumors of the thyroid will yield to this treatment is still unknown. In view of the earlier observations that malignant thyroid tissue often fails to trap iodine, it still remains to be learned how extensively this ingenious mode of therapy may be applied to human cases of thyroid malignancy. At the moment, however, it must be regarded as one of the most hopeful methods of treating cancer of the thyroid after it has metastasized. Fortunately, some metastases which fail to concentrate radioactive iodine early during treatment do so later when the main thyroid mass has been removed or destroyed Only time can give the final evaluation of the procedure.

by which clinicians in a well-equipped clinic may be able to treat thyroid disease advantageously. For example, in serious heart disease complicated by hyperthyroidism the operative risk might be so great that this treatment with radio-iodide would be much safer than surgery. As yet no information is available on the possibility of the subsequent malignant degeneration of tissues so treated Because opotherapy is so readily available, the danger of producing myxedema may not be an important deterent in the use of this therapy Furthermore, it is possible to use conservative doses of radio-iodide and to repeat the treatment if the disease shows exacerbations later.

Following this earlier work hundreds of cases of Graves disease have been treated with radio-iodide. The standard method now used involves the 8-day isotope I^{131}. The dosage employed for a single treatment ordinarily varies between 4 and 10 millicuries, depending chiefly upon the estimated hyperplasia of the thyroid. At the end of a month another dose may be required In some clinics at the present time, the evidence suggests that over 90 per cent of hyperthyroid patients might be treated successfully with radioactive iodide. Only time will tell whether or not this therapy is preferable to surgery.

This same reasoning has been extended by other investigators to the treatment of thyroid malignancy. For example, Palmer and his associates (35) administered radio-iodide to a patient with a metastasis in the thigh bone. They found that this metastasis collected the radio-active material and subsequently decreased in size, with x-ray evidence of improvement in the lesion. This procedure has been continued by Homburger (54) at the Memorial Hospital in New York. An interesting feature of the use of such iodide is the finding that following such therapy the iodine in the circulating blood may mount to unexpectedly

Nevertheless, this protein has a relatively high content of thyroid hormone as compared with plasma. Perhaps the binding of thyroid hormone by the plasma protein is an important factor in the control of the distribution of the hormone to the tissues at large.

How the hormone is distributed across cell membranes is still obscure. Nevertheless, the tissues contain both iodide and protein-bound iodine. The function of this tissue protein-bound iodine remains to be investigated. Perhaps it can be considered as a "thyrenzyme" or "higher enzyme" which intermeshes with other enzyme systems of the cell, notably those systems concerned with the hydrolysis of foodstuffs and those concerned with the oxidation thereof. Both types of enzyme action are enhanced by an excess of thyroid.

The concentrations of thyroid hormone found in blood and other tissues are similar to that of the hydrogen ion. Thus a new physiology based on microcatalytic procedures is being evolved. An important adjunct to this sort of study is the development of ultra-micromethods which permit the measurement of materials in biological fluids or tissues at a concentration of one in a million To this end, the case of thyroid physiology, the advent of radio-active isotopes of iodine has been a fortuitous advantage. Such isotopes may be used to label chemical fractions within the gland and in the tissues Furthermore, radio-iodide has been used to destroy or inhibit the activity of the hyperactive gland in exophthalmic goiter. Thus the endocrine function of iodine is being elucidated by methods which are carrying the study of thyroid physiology deeper and deeper into the biochemical and enzyme field. The great problems ahead lie in the realm of cell physiology.

SYNOPSIS

IN SUMMARY, these chemical studies of the past decade have disclosed many complexities of the "metabolic circuit of the thyroid hormone" (96). This circuit is most readily comprehended by following a hypothetical iodine atom through the devious pathways which it may pursue. In the first instance, the circulating iodide is trapped in the thyroid gland by a colloidal system which allows concentration gradients to be maintained. In short, the ionized iodine atoms change from the free state (I_F) to a colloidally "bound" state (I_B). Thereupon the iodase-like enzymes incorporate the iodine into the tyrosine molecule, thus producing organically combined iodine. Unless a toxic thyroid-blocking agent is present to inhibit the essential enzyme systems, ultimately thyroxine is formed Perhaps the freshly made thyroxine will be released at once; otherwise, it may reside for long periods as a constituent part of the giant thyroglobulin molecules or aggregates which form the colloid stores in the follicles.

From this point, the hormonal iodine starts on its manifold journeys throughout the microcosm. Perhaps it is released as a peptide of thyroxine, small enough to traverse the membrane-barriers of the gland and its blood vessels. Once within the blood-stream, however, it soon associates itself with the plasma protein, and, in particular, with certain characteristic fractions thereof. As the smaller molecules of the plasma proteins leak through the capillary wall they are collected from the interstitial fluid by the lymphatics. The tissues are thus bathed in a medium containing protein which is lower in protein concentration than the blood.

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